

FIG. 1 Schematic representation of the bacterial p53-expression vector

Fig.2 Partial sequence of clone 1 and clone 2

A. Clone 1

GTATGAGGTGGAAGAAACAGAAGTGGTCATNAAGTCATACCAGAAGAACAGCGATCA
5 GGATGVNGHAGACAAAAAGAA----- ~400 bp----
GTATGAGGTGGAAGAAACAGAAGTGGTCATNAAGTCATACCAGAAGAACAGCGATCA --
GGATGVNGHAGACAAAAAGAAAGCTTGGGNNCTATTCTATAGTGTACCTAAAGACT
AGCTTG

10 B. Clone 2

CCTTCCGTTGAGGTATGTTAGTACCCACTGATACCAGTATTGTAAATGACAGACACTGC
TTTCTCTGAGGGGAAGTCTGTTTCATTTTTGCACATTCCCGTTTGTA
----600 bp---CACCACCACACKCACARACACACKCCCCAAAAAAAACAAAAACA
15 AACAAACAAAAAAGCTTGGGCCT

A: Adenosine	R: A or G	K: G or T
C: Cytidine	Y: C or T	N: A, C, G or T
20 G: Guanosine	V: A, C or G	
T: Thymidine	H: A, C or T	

Fig.3 DNA sequence of the mouse mdmx cDNA

1	GTGGCTCTTG	CGAACTCTGG	GTTTGAGAGG	CCGGAAGTGG	TGCTGCCGTT
51	GCTCGCAGTT	TCAAAATGCA	GTGCAGGCCT	TAGGGTCTCC	GGCTGCCACC
101	CCTCCCCCAG	CTAGGAGGGG	GAGCGACTCA	TGGAGCGGCC	GTAAGTTTGC
5	151	TAAGTGTGGA	GTCTTCACTG	CCAAAATGAC	ATCACATTCC
	201	AGTGTTCAGC	ATCTGACAGT	GCTTGCAGAA	TTTCTTCGGA
	251	CAGGTGCGGC	CAAACTGCA	GCTTTTGAAG	ATTTTGCATG
	301	GCAGGGGGAA	GTATTCACCA	TGAAAGAGGT	AATGCACTAT
	351	ATATAATGGT	GAAGCAGCTC	TATGATCAAC	AGGAGCAACA
10	401	TGTGGTGGAG	ATCTTTTGGG	AGATCTACTT	GGATGTCAGA
	451	GAAAGATCCA	AGCCCTCTCT	ATGACATGCT	AAGAAAGAAT
	501	CAGCTTCTAA	TAACACAGAT	GCTGCTCAGA	CTCTCGCTCT
	551	CACACTATGG	ATTTTCCAAG	TCAAGACCGA	CTGAAGCACG
	601	ATACTCCAAT	CCCAGAAAAA	GAAGTGAAGA	AGAGGATACT
15	651	CTACCTCACG	ACATAAATGC	AGAGACTCCA	GAGCAGATGA
	701	GAACATTTAT	CTCAAGATGA	GACATCTAGG	CTTGACCTTG
	751	GTGGGACGTT	GCTGGCCTGC	CTTGGTGGTT	TCTAGGGAAT
	801	ACTGTATTCC	TAAAAGTAAT	GGCTCAACTG	ATTTACAGAC
	851	ATAGGTACTG	CCATTGTTTC	AGACACTACG	GATGATTTGT
20	901	TGAGACCGTG	TCAGAGCAAT	TAGGTGTTGG	AATAAAAGTT
	951	ATTCTGAGCA	AACAAGTGAA	GTAGGGAAAA	CAAGTAACAA
	1001	GAGGTGGGAA	AGGATGATGA	TCTTGAGGAC	TCCAGGTCCT
	1051	TACTGACGTG	GAAGTTACCT	CTGAGGATGA	GTGGCAGTGT
	1101	AGAAGTTTAA	TTCTCCAAGC	AAGAGGTACT	GTTTTCGTTG
25	1151	AGAAAGGATT	GGTATTCGGA	TTGTTCTAAA	TTAACTCATT
	1201	ATCTAATATT	ACTGCCATAC	CTGAAAAGAA	GGACAATGAA
	1251	TTCCCGATTG	TAGGAGAACC	ATTTCACTC	CTGTTGTTAG
	1301	GGATATTTAA	AGGAGGAAAA	GCCCAGGTTT	GACCCTTGCA
	1351	ATTTTTTGAT	TTGGCTCATA	GTTCTGAAAG	CCAGGAGATC
30	1401	CGAGAGAACA	AACAGATATT	TTTTCTGAGC	AGAAAGCTGA
	1451	ATGGAAGATT	TCCAGAATGT	CTTGAAGCCG	TGTAGCTTAT
	1501	GCCTCGGGAT	GGGAACATTA	TTCATGGGAA	GACGAGCCAT
	1551	GTTTCCACTG	TGCCAGGAGA	CTGAAGAAGT	CTGGGGCTTC
	1601	TGTAAGAAAG	AGATTCAGTT	GGTTATTAAA	GTTTTTATAG
35	1651	TCAGTCACAG	AGAAATACTA	GGAGGACCAG	GTCATTTATC
	1701	A			AAAAAAAAAA

Fig 4. Amino acid sequence of the putative mouse MDMX protein, and the alignment with the amino acid sequence of mouse MDM2 protein

| = identical amino acid

: = conserved amino acid

The p53-binding domain is depicted in Bold/Italic
The Zinc-finger motif around position 310-320 and
the putative Ring finger around position 435-480
are indicated in Bold

The putative nucleotide binding site (451-453) is
underlined

	10	20	30	40	50	59
Mdmx	MTSHSTSAQCSASDSACRI	-SSEQISQVRPKLQLLKILHAAGA	QGEVFTMKEVMHYLGQY			
Mdm2	MCNTNMSVSTEGAASTSQIPASEQETLVRPKPLLLKLLKSVGAQNDTYTMKEIIFYIGQY					
	10	20	30	40	50	60
Mdmx	60	70	80	90	100	110
Mdmx	IMVKQLYDQQEQHVMVYCGDLLGDLGCSFSVKDPSPLYDMLRKNLV	TSASNNTDAAQT				
Mdm2	IMTKRLYDEKQQHIVYCSNDLLGDVFGVPSFSVKEHRKIYAMIYRNLV	--AVSQQDSGTS				
	70	80	90	100	110	
Mdmx	120	130	140	150	160	170
Mdmx	LALAQDHTMDFPSQDRLKHGATEYSNPRKRTEEEDTHTLPTSRHKCRDSRADEDLIEHLS					
Mdm2	LSESRQP---EGGSDLK-DPLQAPPEEKPSDDLISRLSTSSRR-RSISETEENTDELP					
	120	130	140	150	160	170
Mdmx	180	190	200	210	220	230
Mdmx	--QDETSRLDLDFE-EWDVAGLPWWFLGNLRNNCIPKSNGSTDLOTNQDIGTAIVSDTTD					
Mdm2	GERHRKRRRSLSFDPISLGLCELREMCSSG-TSSSSSSSSSESTETPSHQDLDDGVSEHSGD					
	180	190	200	210	220	230
Mdmx	240	250	260	270	280	290
Mdmx	DLWFLNETVSEQLGVGIKVEAANSEQ--TSEVGKTSNKKTVEVGKDDDLSDSLSD--D					
Mdm2	CL--DQDSVSDQFSVEFEVESLDSYSLSDGHELSDEDDEVYRVTVYQTGESDTSDFE					
	240	250	260	270	280	290
Mdmx	300	310	320	330	340	350
Mdmx	TDVELTSEDEWQCTECKKFNSPSKRYCFRCWALRKDWYSDCSKLTHSLSTSNITAIPEK-					
Mdm2	GDPEISLADYWKCTSCNEMNPPLPSHCKRCWTLRENWLPD-DKGKDKVEISEKAKLENSA					
	300	310	320	330	340	
Mdmx	360	370	380	390	400	410
Mdmx	KDNEGIDVPDCRRRTISAPVVRPKDGYLKEEKPRFDP CNSVGFLDLAHSSSESQEI ISSARE					
Mdm2	QAEEGLDVPDGKKLTENDAKEPCAEDSEEKAEQTP-LSQESDDYSQPSTSSSIYSSQE					
	350	360	370	380	390	400

Mdmx QTDIFSEQKAE-TESME-DFQ-NVLKPCSLCEKRPRDGNIIHGKTSHLTTTCFHCARRLK
:: ::|||:: ||| :| |:::|| :|| :|||:| |::|::| :|| |::|

Mdm2 SVKELKEETQHKKDESVESSFSLNALIEPCVICGRPKNGCIVHGTGHLMSCFTCAKKLK
410 420 430 440 450 460

Mdmx SGASCPVCKKEIQLVIKFIA
:: :|||:: ||:: :::

Mdm2 RNKPCPVCROPIQMIVLSYFN
470 480

Fig. 5. Nucleotide sequence of the human MDMX cDNA isolated so far.

AATTCGGCACGAGCTAGGATCTGTGACTGCCACCCCTCCCCCACC CGGGCTCGGCGGGGGAGCG
ACTCATGGAGCTGCCGTAAGTTTTACCAACAGACTGCAGTTTCTTCACTACCAAAATGACATCA
TTTTCCACCTCTGCTCAGTGTTCAACATCTGACAGTGCTTGCAGGATCTCTCCTGGACAAATCAAT
CAGGTACGACCAAACTGCCGCTTTTGAAGATTTTGCATGCAGCAGGTGCGCAAGGTGAAATGTT
CACTGTTAAAGAGGTCATGCACTAATTTAGGTCAGTACATAATGGTGAAGCAACTTTATGATCAG
C GGAGCAGCATATGGTATATTGTGGTGGAGATCTTTTGGGAGAACTACTGGGACGTCAGAGC
TTCTCCGTAAAGAACCCAGCCCTCTCTATGATATGCTAAGAAAGAATCTTGTCACCTTTAGCCACT
GCTACTACAGATGCTGCTCAGACTCTCGCTCTCGCACAGGATCACAGTATGGATATTCCAAGTC
AAGACCAACTGAAGCAAAGTGCAGAGGAAAGTTCCACTTCCAGAAAAAGAACTACAGAAGACGATA
TCCCCACACTGCCTACCTCAGAGCATAAATGCATACATTCTAGAGAAGATGAAGACTTAATTGAAA
TTTAGCCCAAGATGAAACATCTAGGCTGGACCTTGGATTTGAGGAGTGGGATGTAGCTGGCCTGCC
TTGGTGGTTTTTAGGAACTTGAGAAGCACTATACACCTAGAAGTAATGGCTCAACTGATTTACA
GACAAATCAGGATGTGGGTACTGCCATTGTTTCAGATACTACAGATGACTTGTGGTTTTTGAAT
GAGTCAGTATCAGAGCAGTTAGGTGTTGGAATAAAAGTTGAAGCTGCTGATACTGAACAAACAAGT
GAAGAAGTAGGGAAAGTAAGTGACAAAAGGTGATTGAAGTGGGAAAAAATGATGACCTGGAGG
ACTCTAAGTCCTTAAGTGATGATACCGATGTAGAGGTTACCTCTGAGGATGAGTGGCAGTGTAC
TGAATGCAAGAAATTTAACTCTCCAAGCAAGAGGTACTGTTTTCGTTGTTGGCCCTTGAGGAAGG
ATTGGTATTCAGATTGTTCAAAGTTAACCATTCTCTCTCCACGTCTGATATCACTGCCATACCT
GAAAAGGAAAATGAAGGAAATGATGTCCCTGATTGTGCGAAGAACCATTTCGGCTCCTGTCTGTTAG
ACCTAAAGATGCGTATATAAAGAAAGAAAACCTCCAACTTTTTGATCCCTGCAACTCAGTGGAATT
CTTGGATTTGGCTCACAGTTCTGAAAGCCAAGAGACCATCTCAAGCATGGGAGAACAGTTAGATAA
CCTTTCTGAACAGAGAACAGATACAGAAAACATGGAGGATTGCCAGAATCTCTTGAAGCCATGTA
GCTTATGTGAGAAAAGACCACGAGACGGGAACATTATTCATGGAAGGACGGGCCATCTTGTCACTT
GTTTTCACTGTGCCAGAAGACTAAAGAAGGCTGGGGCTTCATGCCCTATTTGCAAGAAAGAGATT
CAGCTGGTTATTAAGGTTTTTATAGCATAATGGTAGTACGAACATAAAAATGCATTTATTCAGTT
CACTTACCACATTATTTGAAAATCAATCCTTTATTTAATTTTATTTCCAACCTGTCAGAGAATG
TTCTTAGGCATCAAAATCCAAGGTAGCTGTAAGAAAAATACTGGAGCTAACAATGAAGAACAGAAG
TAATCTGATTAGTCAAATTATTAAGTGCCATGGATTACTTTATGCAGCAGTCAGGTACATAGTT
AGGTGAACCAAAAGAAAACCTTTGAAAACAAGAGATTCTTCCATGCACATTTACAATATTGAGG
TATAATTAACATGATAAAGTGTTTCCTTCTAACGAGTTGTAGAAATCTGAGTAACCAACCAAAAAA
GCAATAGAATGTTTGTGTACCCCCAAAACACTCCCTTCTGCCCCCTCTTCAGACAGTCCTTCAGCTA
TTTCATGGCTCTCACCCTAGTTTTTTTTTTTTTTTTTGCACCTTTTTTTTTTCCGGGGGTATAGGGG
AGGTGTGGGGCGACAGGGTCTGTCTTGTCTGTCTCCCAGGCTGAAGTGCAGTGAGTCAAGATT
GAGCCACTGCACTCCAGCCTGGGTGACAGCGCGAGACTCCATCTCAGAAAAAAAAAAAAAAAAA
AAAAAAACTAT

Fig. 6 Comparison of the amino acid sequences of human MDMX and mouse MDMX.
Each protein consists of 489 amino acids.

```

nMDMX MTSFSTSAQCSTSDSACRISPGQINQVRPKLPLLLKILHAAQAQEMFTVKEVMHYLGQYIMVK
|||||
nMDMX MTSHTSTAQCASDSACRISSEQISQVRPKLQLLKILHAAQAQGEVFTMKEVMHYLGQYIMVK
|||||
hMDMX QLYDQEQEHMVYCGDLLGELLGRQSFVKNPSPLYDMLRKNLVTLATATTDAACQTLALAQDH
|||||
mMDMX QLYDQEQEHMVYCGDLLGDLGCSFVKNPSPLYDMLRKNLVTSAQNNTDAAQTLALAQDH
|||||
hMDMX SMDIPSQDLLKQSAEESSTSRKRTTDDIPTLPTSEHKCIHSREDEDLIENLAQDETSRLDLG
|||||
mMDMX TMDFFSQDRLKHGATEYSNPKRTEEDTHTLPTSRHKCRDSRADEDLIEHLSQDETSRLDL
|||||
hMDMX FEEWDVAGLPWFNLGNLRSNYTPRSNGSTDLOTNQDVGTAVSDTTDDLWFLNESVSEQLGVG
|||||
mMDMX FEEWDVAGLPWFNLGNLRNNCIPKSNGSTDLQTNQDIGTAIVSDTTDDLWFLNETVSEQLGVG
|||||
hMDMX IKVEAADTEQTSEEYGVKVSDDKVIIEVGKNDLIEDSKSLSDDTDVEVTSEDEWQTECKKFNSP
|||||
mMDMX IKVEAANSEQTSE.VGKTSNKKTVEVGKDDDLSDSRSLSDDTDVELTSEDEWQTECKKFNSP
|||||
hMDMX SKRYCFRCWALRKDWYSDCSKLTHSLSTSDITAPE.KENEGNDVPDCRRTISAPVVRPKDAY
|||||
mMDMX SKRYCFRCWALRKDWYSDCSKLTHSLSTSNITA IPEKKNEGIDVPDCRRTISAPVVRPKDGY
|||||
hMDMX IKKENSCLFNPNSVEFLDLAHSSESQETISSMGEQDLNLSEQRTDTENMEDCONLLKPCSLC
|||||
mMDMX LKEEKPR.FDPCNSVGFLDLAHSSESQETISSAREQTDIFSEQKAETESMEDFQNVLKPCSLC
|||||
hMDMX EKRPDGNIIHGRTGHLVTCFHCARRLKKAGASCPICKKEIQLVIKVFIA *
|||||
mMDMX EKRPDGNIIHGKTSHLTTCFHCARRLKKAGASCPICKKEIQLVIKVFIA *
|||||

```

Fig. 7 Comparison of the amino acids sequences of human MDM2 and human MDMX.

```

hMDM2 MCNTNM.SVPTDGAVTTSQIP..AS.EQETLVRPKPLLLKLLKSVGAQKDTYT
hMDMX .....MTSFSTSAQCSTSDSACRISPGQINQVRPKLPLLKILHAAGAQGEMFT
hMDM2 MKEVLGYLGOYIMTKRLYDEKQOHIVYCSNDLLGDLFGVPSFSVKEHRKIYTM
hMDMX VKEVIHYLGQYIMVKQLYDQQEQHMVYCGDILLGELLGCQSFSVKNPSPLYDM
hMDM2 IYRNLVVVNQQESSDSGTSVSENCHLEGGSDQKDLVQELQEEKPSSSHL..
hMDMX LRKNLVTLATATTTDAAQTALALAQDHTMDIPS.QDQLKQSAEESSTSRKRTTE
hMDM2 ..VSRPSTSSRRRAISETEENSDELSEGERQKRHKSDSISLSFDE.....S
hMDMX DDIPTLPTSEHKCIHSREDEDLIENLAQDETSR.....LDLGFEEWDVAGLPW
hMDM2 LALCVIREICCERSSSSESTGTPSNPDLDAGVSEHSGD..WLDQDSVSDQFSV
hMDMX WFLGNLRSNYTPRSNG..STDQTNQDVGTAVSDTTDDLWFLNESVSEQLGV
hMDM2 EFEVESLDSYSLSEEGQELSDDEDVYQVTVYQA.GESDTSFEEDPEISL
hMDMX GIKVEAADTEQ..TSEEVGKVS..DKKVIEVGKNDDLEDKSLSDDTDVEVTS
hMDM2 ADYWKCTSCNEMNPPLPSHCNRCWALRENWLPEDKGKDKGEISEKAKLENSTQ
hMDMX EDEWQCTECKKFNSPSKRYCFRCWALRKDWYS.DCSKLTHSLSTSDITAIPEK
hMDM2 AEEGFDVPDCKKTI...VNSRESCVEENDDKITQASQSQESEDYSQPSTSSS
hMDMX ENEGNDVPDCRRTISAPVVRPKDAYIKKENSCLFNPCNSVEFLDLAHSSSESQE
hMDM2 IIYSSQEDVKEF..EREETQDKESVESLPLNAIEPCVICQGRPKNGCIVHG
hMDMX TISSMGEQLDNLSEQRTDTENMEDC.....QNLLKPCSLCEKRPDGNIIHG
hMDM2 KTGHLMACFTCAKKLKRKPCPVCRQPIQMIVLTYFP *
hMDMX RTGHLVTCFHCARRLKKAGASCPICKKEIQLVIKVFIA *

```


9/9

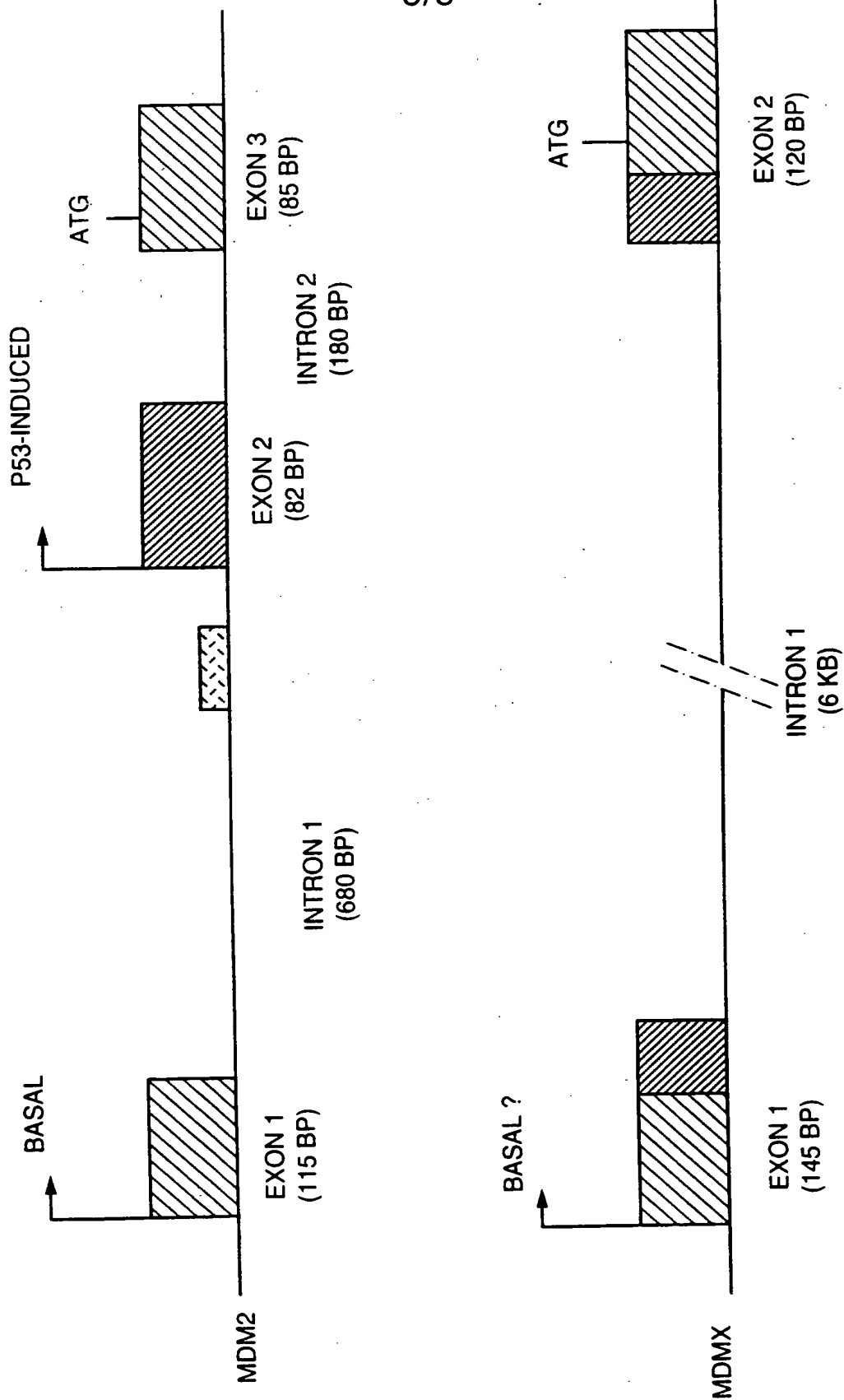


FIG. 8